OBSERVATIONS ON THE CHANGES IN THE HYPODERMIS AND CUTICULA OF COLEOPTERA DURING ECDYSIS.

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The internal changes which occur during the periodic removal and redevelopment of the chitinous portion of the integument of insects are little understood and but few observations have been made thereon. In this paper are given observations and conclusions concerning some of the changes found in the integument of *Leptinotarsa decimlineata* and *Chrysobothris femorata*. These two beetle larvæ are good examples of two types of larvæ, the first living freely exposed upon their food plants and are typical examples of those insect larvæ which pass their lives upon plants in exposed places and must go through ecdysis exposed, and the second of larvæ that live in burrows or cells, protected from external interference to a great degree. Corresponding to the difference in their habitats they show differences in the internal changes accompanying ecdysis.

The life of an insect larva is made up of a series of instars or stages, each of which represents a very precise cycle of development and physiological activities. These cycles of changes are of interest and serve to give a good basis for the orientation of the changes which I am about to describe as accompanying ecdysis. The changes within a single cycle are given in the tabulated form on page 177.

Each larval stage properly begins with the period of growth following the reconstruction period of the preceding ecdysis and ends with the end of the next reconstruction period. Looked at from the exterior the stages extend from ecdysis to ecdysis, but as shown in the above table the period of exuviation is the middle one of the short and rapidly passed over periods in which the process of ecdysis is begun, achieved and the animal recovers from the effects thereof. The changes with which this paper deals are confined largely to the last three periods in the cycle.

Cycle of Periods in one Larval Stage.

Period of Growth.	Period of Maximum Nutrition.	Period of Differentiation.	Period of Preparation.	Period of Exuviation.	Period of Reconstruction.
Larva actively feeding.				Larva not feeding	
Feeding voraciously; rapid increase in size and in thickness of integument; intensification of color.	Feeding voraciously; integument reaches its maximum thickness; abdominal region distended to its full capacity; surface smooth; fat body growing rapidly.	Feeding less actively; abdomen much distended; fat body greatly increased; marked growth of imaginal organs and rapid differentiation of gland cells and leucocytes.	Feeding but little; stops feeding; restless; seeks sheltered place; rapid development of exuvial glands; of zymogens in the hypodemal cells; hypodermis with basement membrane drawn away.	Resting quietly; contractions begin, become rhythmical; dissolving of secondary cuticula; formation of exuvial fluid; rupture of exuvial sutures; detachment of the muscles; formation of new cuticula; removal of old cuticula.	Larva weak; soft; extended; quiet; cuticula hardens; color develops; secondary cuticula begins to form; glands subside; hypodermis becomes condensed into thin layer; larva begins to move about and begins feeding.

The Exuvial Glands.— The existence of an exuvial fluid in insects was first clearly demonstrated by Newport, but its origin remained obscure and was attributed to diverse sources. It was first shown to be in part at least, due to glandular activity by Gonin in *Pieris brassicæ*. Gonin found, especially upon the pronotum, a considerable number of large unicellar glands which were in his preparations definitely seen to be extruding their contents between the old and new cuticula to form a part at least of the exuvial fluid. Similar unicellular glands are found in other insects, especially at pupation, in free living forms such as most of the larvæ of butterflies and moths and of many leaf feeding beetle larvæ. In *L. decimlineata* there are found from 50 to 225 of these glands upon the pronotum in the last larval stage, and their sole function seems to be to develop an enormous amount of the exuvial fluid. The glands are found all through the life of the animal and upon all parts of the body, but in fewer numbers than upon the pronotum at pupation. Some idea of the abundance of these glands may be gained from the section shown in Fig. 1, a section from the pronotum of *L. decimlineata* at pupation when these unicellular glands are closely crowded and are all ejecting their contents into the space between the old and new cuticula (c^1, c^2, c^3).

These glands arise in the embryo and in later larval stages through the modification of the hypodermal cells. In Fig. 2 are shown five stages in the development of these glands.

The first stage in the development of one of these glands in larval life is usually found in the latter portion of the period of maximum nutrition and shows the nucleus greatly enlarged, the chromatin greatly increased in amount and scattered, with the

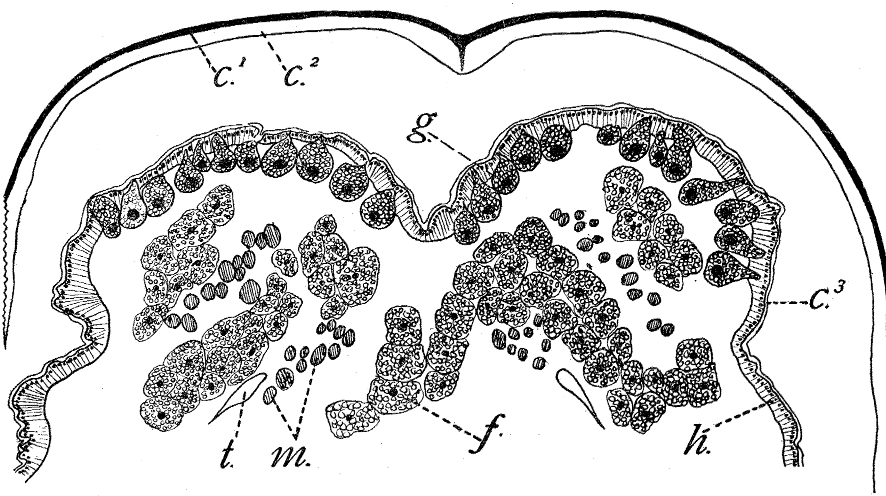

FIG. I. *L. decimlineata*. Section of pronotum of larva about to pupate, in early stage of exuviation, showing large number of exuvial glands, *g*. *C′*, cuticula of larva being removed; C^3, developing new cuticula; *H*, hypodermis; *f*, fat body; *m*, muscles; *t*, trachea.

cytoplasm seen (Fig. 2). In the period of differentiation thisd cell grows very rapidly projecting inward below the hypodermis, but retaining a delicate connection with the cuticula (Fig. 2, *B*). The cell remains in this condition through the following ecdysis and appears after ecdysis unchanged, excepting that the gland now has a delicate duct leading to the surface, this being developed as the result of the glands not forming chitin at this point of attachment to the outer surface of the hypodermis (Fig. 2, *C*), During the last larval stage the character of these glands, especially in the periods of differentiation and preparation, change rapidly (Fig. 2, *D* and *E*), becoming first larger, the nucleus and chromatin growing immensely in size and quantity, the cytoplasm remaining dense and slightly vacuolated. At the begin-

ning of the period of preparation there is a rapid change, chromatolosis of the nucleus, a marked shrivelling and decrease in size. and the cytoplasm becoming entirely vacuolated (Fig. 2, *E*). As ecdysis begins and the old cuticula separates from the hypodermis the contents of these glands are forced out between the old cutic-

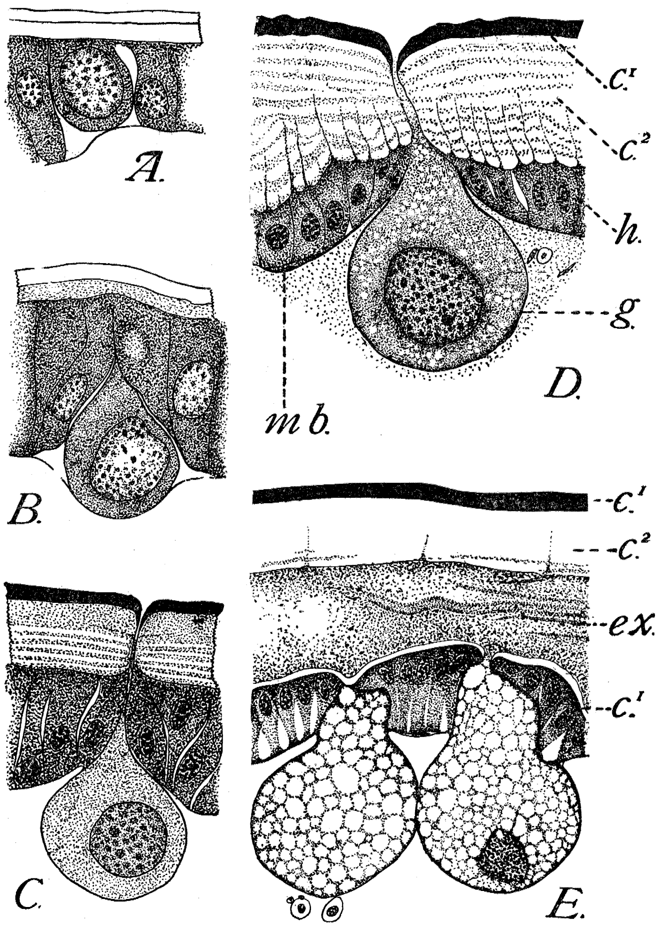

FIG. 2. *L. decimlineata.* Five stages in the development of the exuvial glands. *A*, young stage showing rapid growth of nucleus in hypodermal cell; *B*, later stage showing cell much enlarged and projecting below the hypodermis, taken just before second ecdysis; *C*, third stage just after second ecdysis, showing the cell as a fully developed unicellular gland with outlet to surface; *D* and *E* show the great increase in size of these glands preceding pupation (at *E* is shown glands extruding their contents to form part of the exuvial fluid); *c'*, primary cuticula; *c²*, secondary cuticula; *h*, hypodermis; *mb*, basement membrane.

ula and the new to help form the exuvial fluid. Eventually nearly all of the contents of the glands are extruded, leaving them small and shrunken as shown in Fig. 3, *A*.

After ecdysis and especially after pupation the degeneration of these glands is rapid, as shown in Fig. 3, the cell speedily returning to a normal hypodermal cell in size and ultimately it breaks down completely.

As far as I can discover these glands are exactly like those found by Gonin in *Pieris brassicæ*, and I have observed the same structure in *Pieris rapæ* and *protodice*, and *Clisiocampa americana* among the Lepidoptera and they are widespread in the

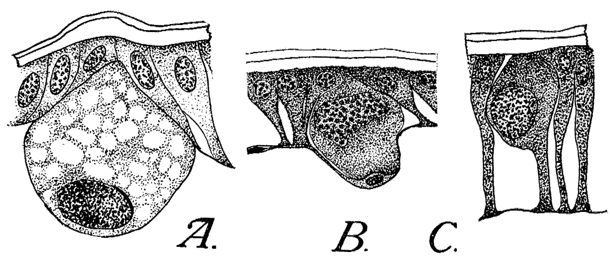

FIG. 3. *L. decimlineata*. Three stages in the degeneration of the exuvial glands. *A*, gland immediately after ecdysis; *B*, stage of gland during the period of reconstruction; *C*, gland nearly reduced to normal hypodermal cell in period of growth.

Chrysomelidæ and Coccinnellidæ, especially in the tropical species. Nowhere have I found these unicellular glands in larvæ that live in burrows, or in the soil or in cells, but only in larvæ living freely exposed upon plants where there exists the greatest liability to rapid desiccation. In *L. decimlineata*, *multitæniata* and their tropical allies these glands developed in the embryo in small numbers over the entire body surface and are active at each ecdysis. As the larva grows, however, the number of these glands increase until at the time of pupation there are very many of them scattered over the body, but they are most numerous upon the pronotum. During the pupal period nearly all degenerate rapidly and but few are functional at the final transformation.

These glands and their increase in number is, I believe, an adaptation in these freely exposed larvæ, to enable them to pass with the least mortality through such critical periods of their life as ecdysis and pupation. I can see no reason why this adapta-

tion would not be one of direct selective value and be greatly developed by selection, because those individuals with an abundant supply of exuvial fluid would have a far better chance of passing safely these critical periods than those with a lesser supply of the exuvial fluid.

Changes in the Integument. — In the integument the preparatory changes preceding ecdysis begin before the larvæ cease feeding and consist largely in the withdrawal of the protoplasmic processes of the hypodermal cells from the pore canals in the secondary cuticula (Fig. 2, *D*) and the gradual change in the shape of the

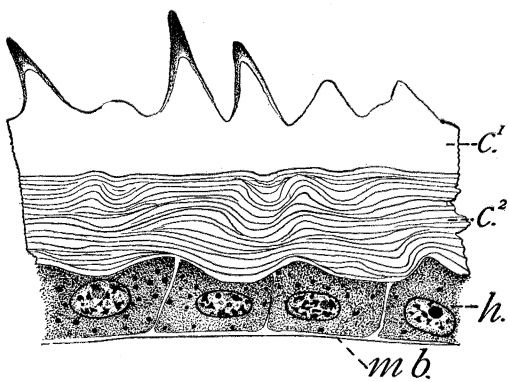

FIG. 4. *C. femorata.* Section in latter end of period of differentiation of last instar, from the mesothorax.

hypodermal cells whereby they become greatly elongated and their outer ends and the basement membrane separated by two, three or four times the usual distance. A section (Fig. 4) shows this condition in which the hypodermis is in the form of a flattened epithelium.

The changes from the condition shown in Fig. 4 go on slowly until at the beginning of the first contractions all the protoplasmic processes have been withdrawn and the hypodermis is much thickened, due to the drawing away of the basement membrane. With the first contractions the old cuticula and hypodermal cells separate over almost the entire body surface and only the muscle attachments remain to hold the old cuticula to the animal.

At the time when the contractions begin or slightly before, a

thin layer of exuvial fluid is found, especially in the anterior parts of the body between the hypodermis and cuticula, and the inner surface of the cuticula appears rough and corroded. This corrosion of the inner side of the secondary cuticula continues until it is often almost entirely removed, as shown in Fig. 5.

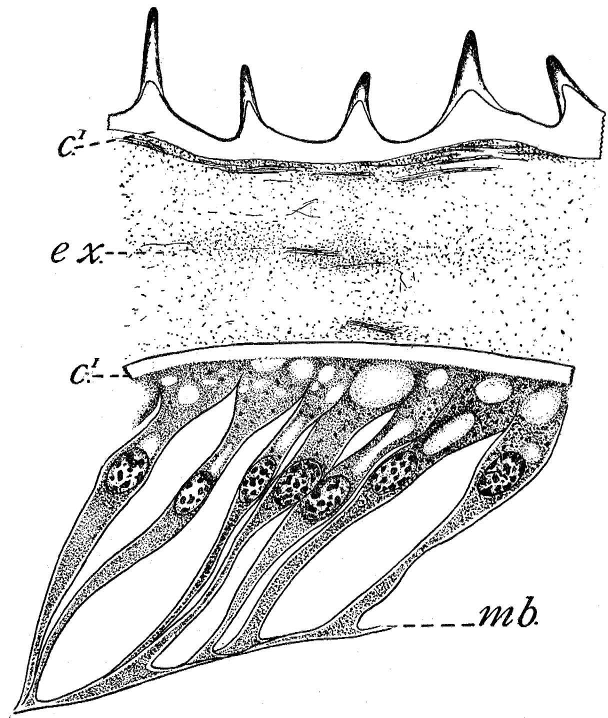

FIG. 5. *C. femorata.* Section of the integument from same location as the section from which Fig. 4 was taken, showing decrease in thickness of the secondary cuticula and great extension of the basal ends of the hypodermal cells to form a relatively thick but open layer of hypodermis.

This dissolving of the secondary cuticula is a most constant phenomenon in ecdysis and has been found in all the insects that I have examined, but in varying degrees. The same disintegration of this layer is shown in Fig. 6 of *L. decimlineata.*

This origin of part of the exuvial fluid in *L. decimlineata* and of all of it in *C. femorata* has not, I believe, been heretofore suspected but we can at once see the great utility of this process and especially the advantage gained in having the cuticula thinned

and softened by the dissolving action which is evidently going on. As far as my experience goes this solution of the cuticula varies greatly in different preparations, being almost but never entirely absent in some and exceedingly active in others. I have not

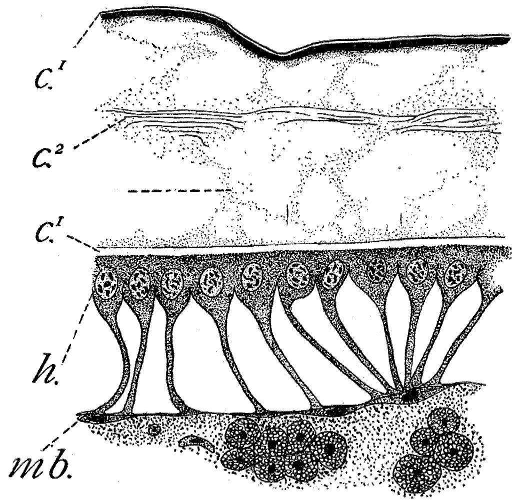

FIG. 6. *L. decimlineata.* Section of pronotum in early part of the period of ecdysis showing greatly drawn out hypodermal cells and almost entirely dissolved secondary cuticula. Fragments of cuticula are floating in the exuvial fluid (C^2).

determined absolutely what brings about this dissolution of the secondary cuticula, but it is probably due to enzyme action. Attempts have been made to isolate these enzymes but thus far without any marked success. I do find, however, in the hypodermal cells in the late part of the period of differentiation and the early part of the period of preparation, granules which react and stain exactly like zymogen granules and which are derived from the nucleus by chromatolosis and which disappear after the action of dissolution of the old cuticula has begun. These may not, however, have anything to do with the disintegration of the old cuticula. It is clear, however, that the action upon the cuticula is chemical as there is not the slightest indication of phagocytes or other organic elements being present. The most logical supposition is that the hypodermal cells secrete a substance that dissolves the old secondary cuticula and thus thins and softens the integument as well as supplying a considerable part of the exuvial fluid, thereby greatly facilitating ecdysis.

These changes which occur in the cuticula involve only the secondary cuticula and are the same irrespective of what the habitat of the larva may be, as shown in the figures given and result in all in the thinning and weakening of the old integument. All these changes take place in the latter part of the period of preparation and the first third of the following stage.

After the separation of the cuticula and hypodermis the new primary cuticula begins to form at once. It appears first as a thin delicate lamella spread evenly over the entire outer surface of the hypodermis and grows rapidly in thickness until finally just before ecdysis takes place it reaches its final thickness. It is developed as a delicate structureless membrane secreted by the hypodermal cells and there appears at no time evidence in favor of the oft-repeated statement that the cuticula is the hardened outer ends of the hypodermal cells. After ecdysis this primary cuticula hardens rapidly and develops its coloration through enzyme action precisely as in the adult beetle, a process which I have described elsewhere.

As soon as ecdysis is over the deposition of the secondary cuticula begins. This layer is, as is well known (Vossler, Tower) a carbohydrate allied to tunicin and is deposited in alternating layers through the periods of reconstruction and growth when it attains its maximum thickness. It is everywhere penetrated by delicate pore canals which are the fine canals occupied by the protoplasmic processes of the hypodermal cells which do not become detached from the primary cuticula until just before ecdysis when they are withdrawn. With poor killing and preservation, however, they are all withdrawn and the canals appear empty, but are not so in life.

The hypodermis also goes through a regular cycle of changes in the shape of the cells during each of the cycles. These changes are first an increase in the number of cells in the growth period, and second changes in shape and arrangement so as to give the body wall the greatest rigidity and strength during the period immediately following ecdysis before the new cuticula hardens. In Fig. 7 I have given a series of stages in semi-diagrammatic form showing the change in the shape and arrangement of the cells. In Fig. 7, *A*, in the growth and differentia-

tion periods the cells are hexagonal flattened epithelial and of themselves would be a relatively weak layer if left alone. In the period of preparation, however, the inner ends elongate, the basement membrane draws away and the cells come to form a much thicker layer, until during the period of ecdysis they present the condition shown in Fig. 7, *D*, which arrangement, even though the thick cuticle be absent, gives a far greater rigidity to the body wall than the arrangement seen in Fig. 7, *A*. After ecdysis is over the cells gradually assume their epithelial character. This series of change represented in Fig. 7 evidently is for no other purpose than that of making the integument as rigid and strong as possible during ecdysis. The arrangement developed is one which in a mechanical way is the best possible under the conditions. In fact the arrangement of the basement membrane, the long drawn out hypodermal cells and the developing cuticle is exactly the system used by engineers in large bridge work or in the building of large spans or girders.

The change in the integument are all in the direction of strength-

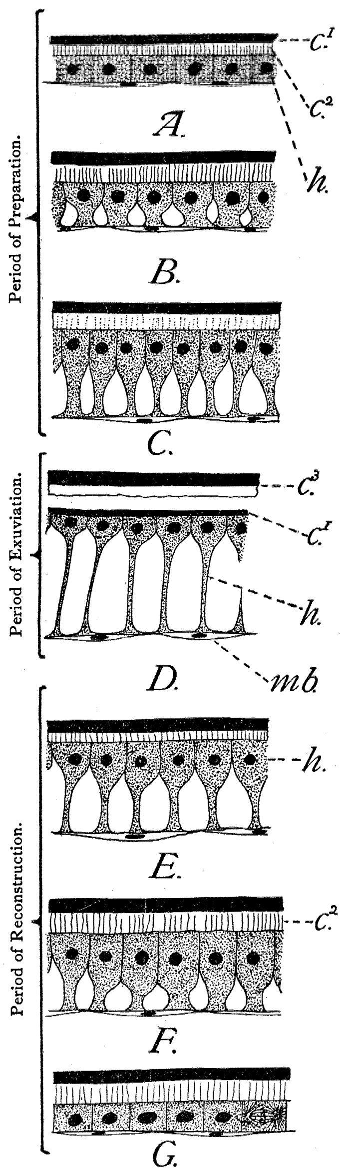

FIG. 7. Diagrammatic representation of stages passed through by the hypodermis before, during and after ecdysis, to show changes in the form of cells. c', primary cuticula; c^2, second cuticula; c^3, old larval cuticula being removed; h, hypodermis; mb, basement membrane. All figures are from *preparations* of *L. decimlineata*.

ening it to enable it to resist the strain and pressure it must undergo during and after ecdysis, and by solvent secretions to weaken the old cuticula and decrease the resistance offered by it to its removal and to provide from the material to be cast away a part of the lubricating fluid, to facilitate the withdrawal of the body from its old cuticula covering.

The Exuvial Sutures. — It is commonly stated that the rupture of the integument during ecdysis is caused by the pressure of the

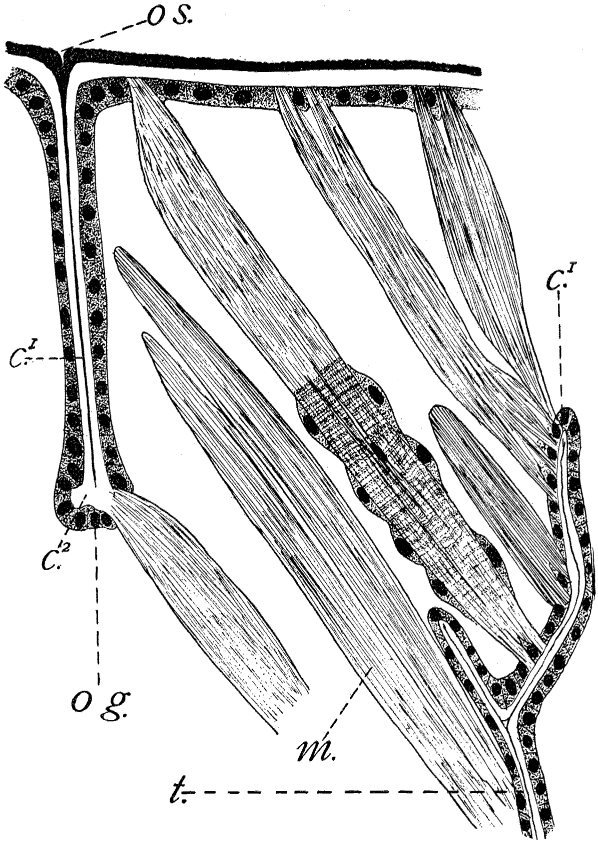

FIG. 8. *L. decimlineata.* Section of the head in the region of the occiput. Last larval instar showing the line of weakness formed in the mid-dorsal line by the inflexed body wall. *os*, occipital suture; *t*, crura of tentorium; *og*, occipital fold.

hæmolymph during the contractions. It is probably true that the pressure of the hæmolymph and the violence of the contrac-

tion aid in this process, but that the rupture should occur so regularly in the mid-dorsal line is remarkable, if this be the only cause of the rupture. In the insects that I have examined there always exists in the mid-dorsal line a special device to aid in the splitting of the old cuticula at ecdysis.

This apparatus in *L. decimlineata* consists of a well-marked laterally flattened invagination extending caudalward from the region of the occipital suture, where it extends deeply into the head (Fig. 8).

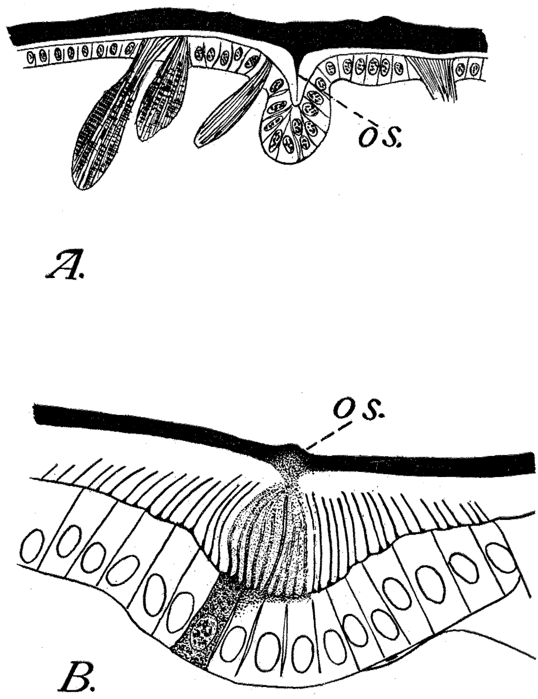

FIG. 9. *L. decimlineata.* Section through mesothorax. (*A*) and second abdominal segment; (*B*) to show continuation of the modifications of median dorsal line of body to permit of rupture at ecdysis.

This line of weakness in the mid-dorsal line is also the last point where the ectoderm closes in in development and is in *decimlineata* turned inward to form a median dorsal fold from the anterior edge of the occiput to the first abdominal segment and in this median dorsal furrow the chitin is easily broken during ecdysis. When the secondary cuticula represented in white in

the figures is nearly dissolved away the muscles are still attached and a slight contraction of the muscles shown in Fig. 8 would suffice to rupture the cuticula in the median line. And that is exactly what occurs. That is, the rupture of the integument is not due to pressure but to the pull of muscles after the cuticula has become softened. The rupture occurs always in the same place because there is there a weak spot in the cuticula and special provision for the rupture thereof.

The same sort of structures are found in *C. femorata*, *P. rapæ*, *C. americana* and I suspect in most insects and it is perfectly clear in all of those examined that the rupture of the integument is as described above and in all it occurs at this same place.

Detachment of the Muscles. — The detachment of the muscles does not begin or progress far until after the formation of the

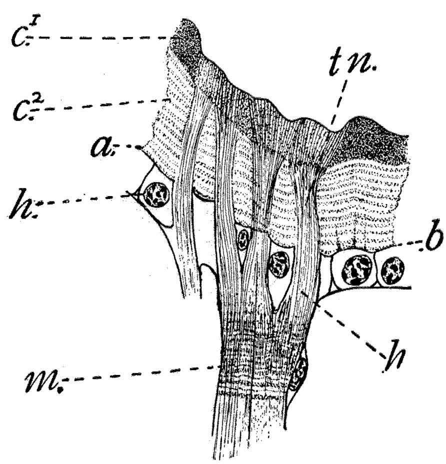

FIG. 10. *C. femorata.* Section of the integument showing the relations of the muscles to the cuticula and the method of the insertion of the muscles. When ecdysis occurs the muscles break from the cuticula along the line *AB*.

exuvial suture and is then accomplished by a few violent contractions aided no doubt by the corrosion of the tendinous portion of the muscle exposed beyond the surface of the hypodermis.

The normal insertion of a muscle in insects to the cuticula is shown in Fig. 10 of *C. femorata*, where the modified tendinous ends are seen to go to the very outer surface of the cuticula. This

seems to be general for insects, almost identical conditions being shown by Holmgren in Lepidoptera.

When the muscle ruptures the break always occurs along the line *AB* (Fig. 10) level with the surface of the hypodermis, and the long drawn out tendinous ends of each muscle are again in-

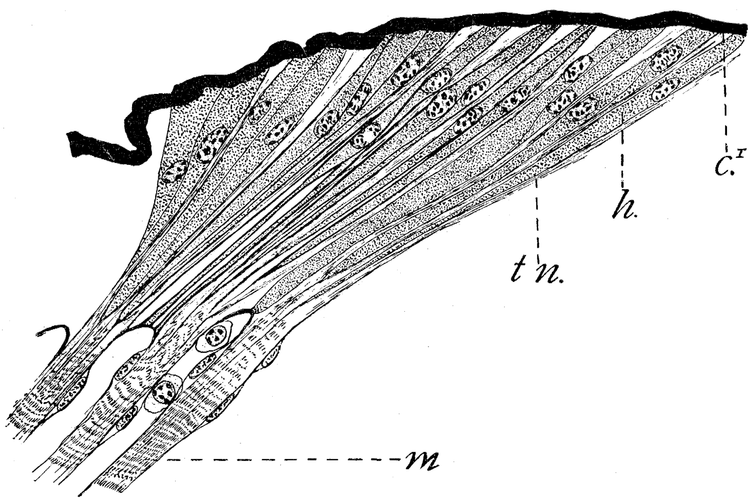

FIG. 11. *C. femorata.* Section to show the relation of the muscle fibers immediately after separation from the old cuticula and after the new cuticula has been partly developed.

corporated into the new cuticula as it is formed, cuticula being developed between the tendons by specialized hypodermal cells and the tendinous ends themselves becoming chitinous.

These muscle changes, as far as they are related to the hypodermis and cuticula behave exactly in Coleoptera as Holmgren has described for the Lepidoptera and the same behavior probably is true for insects in general.

Cuticula Structures Removed and Replaced at Ecdysis. — Considerable diversity of opinion prevails as to what and how much of the chitin is removed at ecdysis in insects. I find in *L. decimlineata* that ecdysis takes place in the following structures:

1. *Body Wall.* — Cuticula entirely removed, including the chitinous covering of all hairs, spines and scales, sensory pits and sense organs, including chitinous lenses of both simple and compound eyes.

2. *Alimentary Canal.* — Entire cuticula lining of both fore and hind gut, including all cuticular structures in the mouth and the linings of the ducts of all the bucal glands.

3. *Tracheal System.* — All tracheal lining of the main tubes and the smaller trachea, but not of the tracheoles. It is possible that the lining of these latter is not chitinous, or is very soft and does not harden and therefore does not need to be removed.

4. *Internal Supporting Structures.* — Tentorium, apodemes and the lining of all ducts and glands, including the reproductive organs or such as exist.

Conclusion. — The observations and figures which I have given show that ecdysis is a more important and far-reaching process in the development of insects than might at first be supposed. Inadequate as are the observations herein recorded, they show changes comparable to other periodic phenomena in animals, in the existence of the periods of preparation, active ecdysis, reconstruction, and quiescence. We cannot of course conclude from this that ecdysis is in any way related to other periodic phenomena in animals, but rather that there exists in all a similarity, due in all probability to the existence of deep-seated laws of growth which control all of these phenomena.

All Arthropods and especially insects cannot grow or further develop without the process of ecdysis, owing to the hard resistant non-extensible chitinous outer portion of the integument. Among insects ecdysis bears a direct relation as far as the specialization of the process is concerned, to the degree of specialization of any particular groups. Thus, in the lower forms like the *Campodea*, according to Grassi, there is a single ecdysis, but this is fragmentary. The integument is shed in small pieces, while in the Colembola the chitin is shed in bits throughout life. A species of *Peripatus* found in the rain forests of Vera Cruz, Mexico, when about one fourth of an inch long did not shed its cuticula at all during its growth. From these primitive conditions of ecdysis found in the most generalized of tracheates and resembling possibly in this function their supposed annelid ancestors, there exist all degrees of specialization up to the most highly developed condition found in Coleoptera and Lepidoptera.

The specialization of ecdysis which goes hand in hand with

the specialization of the integument consists essentially, as I have shown, in the development of a mechanical strengthening of the body wall during ecdysis by the rearrangement of the hypodermal cells in a very precise bridge-like structure ; the arrangement for softening and dissolving a part of the cast chitinous covering, thus decreasing resistance ; and finally the provision of a sufficient fluid lubricator to enable the animal to slip out of its old covering with the least danger of rupturing the body wall, or distortion of the appendages. In these three provisions are features of great utility which present many striking adaptations to the habits and habitats of individual species.

That ecdysis is a crucial period in the life of insect larvæ is the generally accepted belief, but how crucial is quite an open question. During several years in which I have reared larvæ of *Leptinotarsa* for the study of their evolution I have found that ecdysis is one of the phases on insect breeding needing most careful attention. This is especially true in experiments wherein there are used greatly changed conditions of existence, and the failure of many experiments can be attributed directly to improper care and surroundings during ecdysis. Even under normal conditions each recurring cycle acts to eliminate from one to eight per cent. of the larvæ and the average per cent. of individuals in my experiments with *L. decimlineata*, which cover eleven years, that were killed during ecdysis alone, is 13.69 per cent. Often the percentage runs far higher, frequently lower, but even 13 per cent. of a population is a huge death rate to be directly due to one particular process. The mortality from ecdysis occurs largely as the result of failure to rupture the integument, due primarily to its not becoming softened owing to the failure of the cuticula solvents to develop. The larvæ dying from this cause in insect breeding are often passed over as belonging to the death rate of a certain stage, while in reality it is largely the elimination due directly to ecdysis. Comparatively few larvæ die during ecdysis, but about from 1 to 1.5 per cent. become deformed and die in the following stage. Apparently none are killed during the reconstruction stage, excepting by their enemies. If we knew the whole history of say *L. decimlineata*, I think we should be very near to the truth if we hold that in any given population 15 per

cent. are on the average eliminated by the process of ecdysis and the accidents which may occur along with it.

Ecdysis is, in fact, extremely important in the physiological activities of insects, each recurring period being accompanied by great disturbances in all parts and functions of the body besides those described in this paper. Muscles, digestive apparatus and excretory system are deeply influenced by the process, and in some forms at least are the seat of developmental dangers occurring also in rhythmic cycles.

As far as my experience and observations go ecdysis is a peculiar process which periodically reduces various organs and parts of organs to a condition strongly resembling an undifferentiated embryonic condition from which are again built up old or new characters by exceedingly rapid reconstructive processes, each passing through in its development, stages seen in the development of the same or similar earlier larval characters. This repetition of stages in the development of colors or other characters is solely due to the recurrent rhythmic processes necessary to further growth and development. Ecdysis is a process of intense importance in the life of the individual and generation and one upon which natural selection can work most effectively. This preliminary paper suffices to call attention to the importance and deep-seated nature of ecdysis in insect economy, the general features of the internal changes accompanying this process, some of its most striking anatomical changes and its direct selective value in the evolution of insects.

Hull Zoölogical Laboratory,
The University of Chicago,
February 6, 1906.